

RESPONSE OF *CANDIDA ALBICANS*, *C.GLABRATA* AND *SACCHAROMYCES CEREVISIAE* TO CHLORHEXIDINE

*Sarah J. Hiom, *J.R.Furr, *A.D.Russell, **J.R.Dickenson, *Welsh School of Pharmacy and **School of Pure and Applied Biology, University of Wales College of Cardiff, Cardiff CF1 3XF, Wales

Although yeasts and moulds are important pathogens and spoilage organisms, the effects of antiseptics, disinfectants and preservatives are poorly understood. Here, we describe the effects of a bisbiguanide, chlorhexidine diacetate (CHA) on a yeast (*Saccharomyces cerevisiae*), a yeast-like fungus (*Candida albicans*) and a yeast that does not form pseudohyphae (*C.glabrata*). Strains consisted of *Sacch. cerevisiae* A 364A (wild type), its mannoprotein mutants LB1 - 10B (defective $\alpha 1 - 3$ -mannosyl-transferase), LB1 - 3B (defective $\alpha 1 - 2$ -mannosyltransferase-II) and LB 6 - 5D (mannoprotein is unphosphorylated) and its permeability mutants 2512C - 2A (defective general amino acid permease) and RA68 (all obtained from the University of California, Berkley, U.S.A.); *C.albicans* NCYC 1363 and *C.glabrata* NCYC 388. Minimum inhibitory concentrations (MICs) were determined after incubation for 24hr at 30°C in Sabouraud Broth (Oxoid). Inactivation of *Sacch. cerevisiae* A 364 and the *Candida* strains were measured when cells (initial density ca. $5 - 10 \times 10^6$ cfu/ml) were exposed as washed suspensions to different CHA concentrations at 30°C; excess CHA was quenched by dilution in Sabouraud broth containing 0.75% Azolectin and 5% polysorbate 80 and colonies counted after incubation of Sabouraud agar plates for 24 hr at 30°C.

MICs of CHA were 10 µg/ml against *Sacch. cerevisiae* A346A, 4 and 8 µg/ml for RA 68 and 2512C-2A, respectively and 16, 8 and 8 µg/ml respectively against the mannoprotein mutants LB6-5D, LB1-10B and LB1-3B. The mannoprotein mutants are being studied to determine the effect of the mannan content of the yeast cell wall on chlorhexidine sensitivity. It is not as yet possible to reach a definite conclusion although MIC values suggest it has only a minor role. *C.glabrata* was inhibited by 20 µg/ml and *C.albicans* by 20-30µg/ml (in the presence of 200 µg/ml mercaptoethanol, this was reduced to 10-15µg/ml). All these organisms are inhibited by CHA concentrations that are much higher than those (1-2µg/ml) needed to prevent bacterial growth (Russell & Gould 1988). The decreased sensitivity of yeasts, however, suggests either a cell wall permeability barrier and/or reduced plasma membrane sensitivity to CHA. CHA was fungicidal (Table 1) although *Sacch. cerevisiae* was killed more rapidly and to a greater extent than the two *Candida* strains.

Table 1. Fungicidal activity of chlorhexidine diacetate (CHA)

Organism	CHA concn.	Fungicidal activity
<i>Sacch.cerevisiae</i> A364A	10	2 log reduction, 4 min
	100) >6 log reduction, <20 sec
	1000	
<i>C.albicans</i>	10	No loss of viability
	100	2 log reduction, 3.5 min
	1000	2 log reduction, <20 sec
<i>C.glabrata</i>	10	No loss of viability
	100	2 log reduction, 5.5 min
	1000	2 log reduction, <20 sec